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SOME FACTORS AFFECTING THE SEVERITY OF THE ROOT-ROT COMPLEX OF SUGAR CANE

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Factors Influencing the Severity of the Root Rot Troubles of Sugar Cane

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FACTORS INFLUENCING THE SEVERITY OF THE ROOT ROT TROUBLES OF SUGAR CANE

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INTRODUCTION

IN THE HISTORY of the sugar cane industry, the intermittent replacement of old varieties with new ones forms one of the important chapters in most sugar producing countries. In most regions, the industry was started with some of the noble canes, such as Bourbon, Otaheite, or Lahaina, but after a time, on account of declining yields, these were abandoned. While the failure of these canes has usually been more or less gradual, sudden crop failures followed by financial crises have occurred in some countries. Such a crisis occurred in the islands of Mauritius and Bourbon as early as 1846. Another occurred in the Mayaguez district of Porto Rico in 1872. Jamaica and other islands in the British West Indies have also had similar crises. In Cuba, the Cana Blanca (Otaheite) variety has been almost completely abandoned, but the change to other varieties has been gradual on account of the large areas of virgin lands suitable for cane culture. In Hawaii, the Lahaina has been almost completely replaced by H 109 and other varieties, while in Java there have been several changes of varieties.

During recent years, the sugar industry of Louisiana has passed through a crisis in many ways similar to those which had occurred in the tropics. Beginning about 1908 or 1909 there was a gradual decline in the yield of sugar per acre until about 1923. Previous to 1909, the acre yield of sugar in Louisiana had been about 3,000 pounds, but this decreased to about 2,000 pounds during the following

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fifteen years. After 1923, due to the presence of the mosaic and other diseases and the occurrence of several seasons with adverse growing conditions, the average sugar yield again took a sharp decline. From 1923 to 1926, the cane crops of the state were practically failures. The D 74 and the so-called native varieties, Louisiana Purple and Striped canes, were unable to make satisfactory yields under the conditions existing on the plantations. Sugar production reached its lowest point in 1926 when 68,000 tons of sugar were produced, as compared with a 10-year average (1911-1920) of 230,000 tons. During this period of depression, certain Java seedlings capable of growing well under Louisiana conditions were introduced and propagated. By the fall of 1929, most of the acreage was planted with these canes.

Many theories have been advanced from time to time to explain the declining yields of sugar cane varieties. The problem is extremely complex and undoubtedly in the final solution many different factors must be considered. One of the factors which has played an important role in decreasing cane yields in many countries is the disease or group of diseases commonly called root rot, root disease, root rot complex, and growth failure complex. In this bulletin, only this phase of the problem is considered. The root rot problem includes the following: (a) The possibility of a physiological deterioration of the cane itself; (b) the influence of nutritional and physical soil condition on cane growth; (c) the rotting of the roots by fungous parasites and (d) the effect of nematodes and other soil animals that attack the root system of the cane plant.

Historical

In regard to the theory that reduction in yield may be due to senility or a physiological deterioration of a variety, it has been proved quite conclusively by Lyon (30) in Hawaii that the failure to grow does not rest in the cane itself. By transferring severely affected stools from Waipio, where the root rot trouble was severe, to Honolulu,

where it did not occur, he demonstrated that the Lahaina variety had not lost its ability to grow and produce large and vigorous canes. He stated that "by the end of the summer, these stools had produced a perfect stand of as healthy Lahaina cane as could be found anyhere in the Islands."

The importance of physical and nutritional soil factors and their relation to the growth failure problem has been studied by a number of investigators. Sorauer (38) stated that the experiments of Kammerling confirmed beyond all doubt that the root rot of sugar cane was a constitutional disease caused by a compacting of the soil. As the disease was most severe in heavy clay soils, the addition of humus was recommended as a corrective. McGeorge (32) recognized the similarity between the root rot or Lahaina disease of cane in Hawaii and a root rot of corn in the United States which had been described by Hoffer (22). He found that salts of aluminum in the concentrations present in many acid soils had a toxic action upon the growth of sugar cane. Varieties differed in their susceptibility to aluminum toxicity. Potash in large quantities and phosphate were correctives, whereas, lime gave little or no immediate response. Bird (3) reported that the epidemics of dying cane, which have been periodic in Demerara, have been attributed to various root diseases, but in all probability their prime cause has been the toxic effect of excessive magnesia.

The role played by fungi has been the dominant phase in most investigations on the growth failure problem. Earle (11) reports that Treub (41), in 1885, referred at some length to a Pythium as a possible cause of sugar cane root rot in Java. Wakker (44, 45), however, also working in Java, seems to have been the first to assign a definite cause for the disease. Although he mentioned the occurrence of Pythium, he concluded that a species of Marasmius was the principal cause. Wakker's work seemed to have influenced that of other investigators as root rot was later ascribed to *Marasmius sacchari* by workers in Barbados

(24, 29), Cuba (23), Hawaii (8), and Porto Rico (26). Other organisms that have been held responsible, at least in part, for root rot are: *Marasmius plicatus* in Louisiana (12, 18); *Ithylallus corallodies* in Hawaii (8); Rhizoctonia species in Porto Rico (33), Barbados (4), and Louisiana (14, 15, 40); and Pythium in Porto Rico (33), Hawaii (5, 6, 7), and Louisiana (14, 15, 16, 40). The investigators in Louisiana found that *Marasmius plicatus* was often present on plants affected with root disease but extensive inoculation experiments in the field of greenhouse did not show that it was capable of injuring cane seriously. They also found that certain strains of Rhizoctonia are capable of rotting cane roots in sterilized soil, but decreased yields were not obtained consistently in field inoculations. They also isolated a number of strains of Pythium from cane roots, some of which were found to be actively parasitic on cane roots, others mildly parasitic, while a few were practically non-parasitic. The Pythium strains which were parasitic on cane were also able to attack other members of the grass family including corn, wheat, oats, sorghum, and rice. Recently, Drechsler (10) has described a Pythium from corn roots as a new species, *Pythium arrhenomanes,* and Johann *et al* (25) have shown that this Pythium is responsible for a serious corn root rot, especially in wet soils at low temperatures. The identity of *Pythium arrhenomanes* with strains found on sugar cane roots was not established.

Nematodes and other soil inhabiting animals have also been studied in connection with the root rot problem. In 1889, Kruger (27) reported a gall forming nematode, *Heterodera radicicola* (Muller), and a non-gall forming one, *Tylenchus sacchari* (Saltwedel), on the roots of sugar cane in Java. In 1908, Cobb (8) reported the root knot nematode and *Tylenchus biformis* (Cobb), later found to be *Tylenchus similis* (Cobb) (9), in the roots of sugar cane in Hawaii. Matz (34) reported a gall forming nematode injuring cane in loose sandy soils in Porto Rico. Muir and Henderson (35) reported the infection of sugar cane roots

in Hawaii by *Heterodera schachtii* (Schmidt) in addition to the two species reported by Cobb.

The pitting and eating of sugar cane roots by soil animals has been commonly observed in most fields where sugar cane is grown. Rands (36), in 1924, brought out the possible relation of root pitting to the root rot situation in Louisiana. He attributed this pitting of sugar cane roots to a small snail, *Zonitoides arboreus* (Say), and thought that the pits made possible the entrance of root rotting fungi. Later (37), he found two centipedes contributing to the damage formerly attributed to snails. Van Zwaluwenburg (43) found a Collembolous insect (Isotomodes) and two species of centipedes primarily concerned with root pitting in Hawaii. Barnum and Van Zweluwenburg (2) found that the growth of cane was reduced by either Isotomodes or Pythium, but that the loss was more pronounced when both were present. The Isotomodes which feed on young tissues caused a great reduction in the number of secondary and tertiary roots. Spencer and Stracener (39) in controlled experiments were unable to verify Rands' observations that snails played the most important role in the pitting of cane roots in Louisiana. These investigators found pitting was due primarily to the feeding activities of Collembolous insects and, to a less extent, Symphylids. The destruction of secondary and tertiary rootlets seemed to be more important than pitting. Edgerton and Tims (15) found that pitting occurred very extensively throughout the sugarcane area of Louisiana. They made a detailed examination of a large number of pitted cane roots and found that a great majority of the pits extended only to the central stele, and that less than five percent penetrated the pericycle.

Symptoms of Root Rot

The terms root rot and root disease have been used in various sugar cane producing countries to refer to any failure of the plants to grow as they should on account of the failure of the roots to function normally. As many

factors influence the growth of the plants and the normal functioning of the roots, the terms, *growth failure complex,* as used in Hawaii, and *root rot complex,* as used in Louisiana, are more descriptive.

The characteristics of plants affected with root rot vary to a certain extent. The most evident symptom is the failure of the plants to grow normally. Throughout the spring and early summer, the affected plants have a stunted appearance, fail to stool normally, and are of a light green or yellow color. In advanced stages, the tips and margins of the leaves often turn brown and die. The roots are shortened, greatly reduced in numbers and rotted. In severe cases, the root system is so deficient that the whole stool may be pulled from the ground with little effort.

Statement of the Problem

From the nature of the symptoms it is evident that anything which affects the development or functioning of the roots becomes a factor in the root rot problem of sugar cane. As many factors may affect the root system of cane plants, it can be seen that the active factors in one country may not be the same as in another.

At the Louisiana Agricultural Experiment Station, there has been in progress for a number of years an extensive study of the root rot problem. Articles (15, 16, 40) already published have shown the relation of certain parasitic fungi, especially species of Pythium, to the problem and have brought out the importance of other factors.

In the present paper, certain phases of the root rot problem will be treated. These will include:

1. Studies on possible root rot causes other than the attack of parasitic fungi. These include the deterioration of the cane, accumulation of toxic substances in the soil, and the action of nematodes and other soil animals.

2. Studies of environmental conditions, such as temperature, moisture, and hydrogen-ion concentrations, which influence the activities of various parasitic organisms, including the fungous parasites.

3. Studies on the physiology of infection by Pythium.

Deterioration of Cane

To test out the possibility of a physiological deterioration of cane and to determine whether plants suffering from root rot have lost their vitality or ability to grow, some tests were conducted in the fall of 1928. Healthy and severely diseased stalks of Louisiana Purple cane were obtained and separated into below ground (rhizomes) and above ground (tops) portions. These were planted in 10-quart pails filled with soil which had been taken from the field beneath diseased stools. In part of the pails, the soil was left untreated, and in the rest it was steamed for two hours at 15 pounds pressure. The soil was kept at a moisture content of 60 percent of its water-holding capacity. Three eyes were planted in each pail and two pails of healthy cane and five of diseased cane were used. Growth through the winter months was slow. The results of the measurements made in March, five months after planting, are given in table 1.

TABLE 1.

GROWTH OF DISEASED AND HEALTHY LOUISIANA PURPLE CANE IN STEAMED AND UNTREATED CANE-SICK SOIL.

Cane planted	Steamed Soil			Untreated Soil		
	Number of eyes	Average % germination	Av'age height of plants in inches	Number of eyes	Average % germination	Av'age height of plants in inches
Healthy rhizomes	6	50	44.5	6	50	33.0
Diseased rhizomes	15	80	40.2	15	27	25.0
Healthy tops	6	83	38.0	6	83	24.0
Diseased tops	15	80	39.2	15	87	21.6

The results which are similar to those obtained by previous investigators indicate that there is no physiological deterioration of the cane itself. The top buds from diseased and healthy stalks produced plants which grew equally well, though both made a decidedly better growth in the steamed soil. (Fig. 1). Plants developing from the

FIG. 1. Comparison of Louisiana Purple cane growing from top seed pieces taken from healthy and root rot affected stalks. Pail 1—Seed from healthy plant, in steamed soil. Pail 2—Seed from a healthy plant, in untreated soil. Pail 3—Seed from a plant severely affected with root rot, in steamed soil. Pail 4—Seed from a plant severely affected with root rot, in untreated soil.

rhizome buds of diseased stalks grew nearly as well as those from healthy stalks in the steamed soil, but in the untreated soil, the growth was poor, and many plants died (Fig. 2). This is in agreement with the field observations that poor stands often occur in the stubble crop, due to the fact that many cane stools suffering from root rot die during the winter.

ACCUMULATION OF TOXINS IN THE SOIL

With the rainfall of 55-60 inches, which normally occurs in the Louisiana cane belt, there is little chance under good drainage conditions for an accumulation of soluble toxic compounds in the soil. However, when the rivers are in flood stage, seepage spots often develop in the cane fields. Water is forced through underground channels to the seepage areas, where it rises to the surface. Here the soil surface is continually wet. If the water carries any

FIG. 2. Comparison of Louisiana Purple cane growing from rhizomes taken from healthy and root rot affected stalks. Pail 1—Rhizome from healthy plant, in steamed soil. Pail 2—Rhizome from healthy plant, in untreated soil. Pail 3—Rhizome from a plant severely affected with root rot, in steamed soil. Pail 4—Rhizome from a plant severely affected with root rot, in untreated soil.

appreciable quantity of salts or if toxic substances are produced in the soil, these could accumulate in the surface layer because of continual evaporation. Cane growing in seepage areas usually shows severe symptoms of root rot.

Inorganic salts which might accumulate in the surface layer of the soil would include, among others, sulphates of various metals. As certain of these sulphates are known to be toxic in certain soils, a greenhouse experiment was conducted to determine if similar results could be obtained with Louisiana cane soils. The sulphate salts of iron, aluminum, calcium and magnesium were added to a cane soil in six-inch pots in amounts varying from 0.5 to 20 times the molecular weight per million parts of soil. In this treated soil, D 74 cane was planted and allowed to grow for four months during the winter season.

The results obtained indicated that none of the salts was toxic in the proportions used. In Hawaii, McGeorge (32) had reported that culture solutions containing alumi-

num sulphate were toxic to root growth of cane. As 60 times the amount used in the Hawaiian experiment was added to the Louisiana soil, it is quite probable that aluminum toxicity is not an important factor in Louisiana. This soil was slightly alkaline (pH 7.8) in reaction and this factor alone precludes the chance of aluminum toxicity.

Another experiment was conducted in the fall of 1927 to determine if there were any soluble toxin present in the soil. Soil obtained from a seepage spot on which cane showed severe symptoms of root rot was taken to the greenhouse and divided into three lots. The first lot was thoroughly washed by filling pails with half soil and half water. The soil and water were stirred thoroughly and after settling over night, the water was poured off. This process was repeated six times. The soil was then air dried until of good tilth. The second lot was treated with live steam for two hours, and the third lot left untreated. The water-holding capacity of the untreated soil was obtained, using the method devised by Hilgard (21). After a thorough mixing, the soil was put in galvanized pails, each pail receiving the equivalent of 18 pounds of oven dried soil. The pails were divided into four series and enough water added to bring the moisture content to 25, 50, 75, and 100% of the moisture-holding capacity. Three single eye pieces of Louisiana Purple cane were planted per pail and four replications of each treatment were made. The results obtained are given in Table 2.

TABLE 2.

GROWTH OF LOUISIANA PURPLE CANE IN WASHED AND STEAMED SOIL. GREENHOUSE JANUARY 24 TO APRIL 10, 1928.

Percent of the water-holding capacity of the soil	Washed Soil		Untreated Soil		Steamed Soil	
	% germ.	Average height in in.	% germ.	Average height in in.	% germ.	Average height in in.
25%	67	12.1	25	5.7	17	12.0
50%	92	19.7	83	23.9	75	34.0
75%	58	16.0	75	20.4	92	40.0
100%	42	9.1	33	11.0	63	15.6

The superiority of plants grown in the steamed soil and the similarity of those grown in the washed and untreated soils indicated that soluble salts or toxins were not primarily concerned in the root rot problem.

Both extremes of moisture used in this experiment were beyond the range favorable for plant growth. Cane grown in the washed soil at 25 percent of the water-holding capacity was better than that in the untreated and steamed soils, because, in washing, a considerable amount of silt and clay was removed. This gave the plants more available moisture, as the water-holding capacity was based on untreated soil. The plants in the steamed soil of the intermediate moisture contents (Figs. 3 and 4) were not only nearly twice as tall as those growing in the untreated and washed soils, but also were more vigorous and of a deeper green color. Roots in the steamed soil were healthy, while those in the washed and untreated soils were badly rotted. The best growth in the washed and untreated soils was obtained in the 50 percent moisture series, while that in the steamed

FIG. 3. Growth of Louisiana Purple cane in washed, untreated and steamed seepage soil, held at 50 percent of its water-holding capacity. Greenhouse, January 24 to April 10, 1928.

FIG. 4. Growth of Louisiana Purple cane in washed, untreated, and steamed seepage soil, held at 75 percent of its water-holding capacity. Greenhouse, January 24 to April 10, 1928.

soil was at 75 percent. This indicates that cane grows best at a relatively high soil moisture content when the soil is free from parasitic organisms; but when parasitic organisms are present, a lower moisture content of the soil is more satisfactory.

NEMATODES

A survey of the cane producing regions of Louisiana has revealed two types of nematodes inhabiting cane roots. One, the gall forming root knot nematode, *Heterodera radicicola*, was found very sparingly in widely scattered regions in sandy soil, while the other which has not been identified, but is similar to *Tylenchus similis* (Cobb) was found to be almost universally distributed, though no cases of severe infestation were found. Attempts to obtain these nematodes in pure culture were unsuccessful.

The effect of variations in soil moisture on the develop-

ment of root knot of sugar cane was studied in the greenhouse. It was found that the root knot nematodes from cane readily infested tomato. In order to preclude to some extent the effects of other organisms which might be found on cane roots, galled roots of tomatoes that had been inoculated with nemas from cane were used as inoculum. A mixed soil sterilized for two hours at 15 pounds pressure was used. Three single eye pieces of Louisiana Purple cane were planted in each 10-quart pail of soil. Four pails were used at each moisture variation. To obtain the effect of temperature, one series was planted in July and another in October. The data obtained from these tests are given in Table 3.

TABLE 3.

EFFECT OF MOISTURE ON GROWTH OF LOUISIANA PURPLE CANE IN SOIL INFESTED WITH THE ROOT KNOT NEMATODE, HETERODERA RADICICOLA.

Percent water-holding capacity of the soil	AVERAGE HEIGHT OF STEMS IN INCHES			
	July 18 to Sept. 13, 1927		Sept. 26 to Dec. 11, 1927	
	Check	Heterodera	Check	Heterodera
25	5.3	5.8	6.5	4.7
45	9.0	7.9	9.1	8.0
65	8.6	8.7	8.5	7.7
85	11.7	11.4	8.2	8.1

The results indicate that injury due to the root knot nematode increases as the water content of the soil decreases. While typical galls were found on inoculated plants at all moisture variations (Fig. 5), an examination of the roots after the soil had been washed away, showed that they were more abundant on the plants grown in soils kept at the intermediate moisture contents than on those growing in the wettest or driest soils. Galls were most prevalent on the tips of both primary and secondary roots. Roots so affected ceased growth, and this stunting of the roots seemed to be the principal form of injury.

Fig. 5. Gall development on roots of Louisiana Purple cane produced by nematode, *Heterodera radicicola*. Plants grown in nematode infested soil and held at 25, 45, 65 and 85 percent of its water-holding capacity. Greenhouse, September 26 to December 13, 1927.

Soil Animals Other Than Nematodes

Another phase of the sugar cane root rot problem is that of injury to the roots by soil inhabiting animals. The most evident form of this injury is the presence of small pits which penetrate directly into the root. These pits are usually circular in shape and most of them do not penetrate beyond the cortex. Not more than five percent (15) of the pits extend into the stele. At first the pits are clean cut and show little discoloration, but later the tissue around them turns a reddish or brownish color. Pitting occurs universally throughout the sugar cane area of Louisiana and freshly made pits have been observed at all seasons of the year except during cold weather. A less conspicuous form of injury, due to soil animals, is the eating off of the root tips, especially those of the secondary and tertiary roots. While a number of soil animals may be concerned with root injury, there is evidence (39) that the Collembolous insect, *Lepidocyrtus violentus*, commonly called springtail, is the most important. Besides the direct injury to the roots, it has been suggested that the pits may serve as avenues of entrance for root rotting fungi, such as Pythium.

Experiments to determine the relative importance of root pitting insects and a parasitic species of Pythium were conducted during 1928. Sugar cane was grown at

different seasons of the year in the greenhouse in 10-quart pails of steamed soil. Some of these were left uninoculated, while others were infested or inoculated with *Lepidocyrtus violentus* alone, *Pythium sp.* alone, and *Lepidocyrtus violentus* and *Pythium sp.* together. Two series were completed, one in the spring and the other in the summer. Four pails of each treatment were used and three single eye pieces of Louisiana Purple cane planted in each pail. Pythium inoculations were made immediately before planting by thoroughly mixing a three-day-old culture of a parasitic Pythium with the soil. The *Lepidocyrtus violentus* infestations were made by adding 50 of the insects to each pail immediately after planting. The soil was kept at about 60 percent of its water-holding capacity by weighing the pails regularly. The results are given in Table 4.

TABLE 4.

THE INFLUENCE OF PYTHIUM AND LEPIDOCYRTUS VIOLENTUS (Folsom) ON THE GROWTH OF LOUISIANA PURPLE CANE IN STERILIZED SOIL IN THE GREENHOUSE.

Treatment	Feb. 7 to Apr. 11, 1928			July 7 to Sept. 17, 1928		
	Percent germination	Length of stem in inches	Percent of plants stooling	Percent germination	Length of stem in inches	Percent of plants stooling
Check	92	7.0	0.0	92	13.7	75
Lepidocyrtus violentus	92	6.1	0.0	100	13.8	75
Pythium	100	3.2	0.0	83	12.1	60
Lepidocyrtus violentus and Pythium	75	2.3	0.0	92	10.7	33

The results of these tests indicate that root pitting animals, such as *Lepidocyrtus violentus,* may retard cane growth and aggravate injury due to Pythium. Their influence on growth was greater under the cool growing conditions of spring than under those of summer. An examination of the soil and the roots showed that the insects had increased greatly in numbers, and although there was not a great deal of pitting, there was a decrease in the number of secondary roots (Figs. 6 and 7).

FIG. 6. Effect of springtails, *Lepidocyrtus violentus*, and Pythium on the growth of Louisiana Purple cane in steamed soil held at 60 percent of its water-holding capacity. Greenhouse, February 7 to April 10, 1928.

SOME FACTORS INFLUENCING THE PARASITISM OF ROOT ROTTING ORGANISMS

While it has been demonstrated quite conclusively that certain fungi, especially species of Pythium and Rhizoctonia, have the ability to attack and rot roots of sugar cane and corn, the conditions favorable or unfavorable for this attack have not been definitely established. Previous experiments in Louisiana (16) have shown that root rotting species of Pythium are abundant in most of the cane soils, yet, the severity of the root rot disease varies to a great extent from year to year. These organisms are known to be facultative parasites, living readily on both dead organic matter and living plants. Their ability to seriously injure the host seems to depend upon environmental conditions, as well as upon the thriftiness or vitality of the latter. To obtain some idea of the conditions which favor or retard the action of these organisms, a study has been made of the effect of moisture, temperature, and soil acidity on both parasite and host.

FIG. 7. Effect of springtails, *Lepidocyrtus violentus*, and Pythium on root development of Louisiana Purple cane in steamed soil held at 60 percent of its water-holding capacity. Greenhouse, February 7 to April 10, 1928.

MOISTURE AND TEMPERATURE RELATIONS

The sugar cane plant is a native of the tropics and is subjected to abnormal weather conditions when grown in Louisiana. These conditions may influence the crop directly by the effect on the growth and development of the cane plant, or indirectly, by providing favorable conditions for the development of parasites.

McDonald (31) has shown that high yields of sugar per acre in Louisiana are directly correlated with high winter temperature, especially in March, and low winter rainfall; while Edgerton and Tims (15) have shown that high yields have followed winters (January, February and March) of low rainfall, and low yields winters of high rainfall. It has also been frequently observed that the

effects of root rot are more noticeable in dry summers following wet winters. In seasons of ample rainfall, even a deficient root system is able to absorb sufficient water for plant growth.

In order to test under controlled conditions the effect of soil moisture and temperature on the severity of root rot as produced by the important root rotting fungi, Pythium and Rhizoctonia, a number of tests were conducted in the greenhouse and laboratory. These were run with both cane and corn. As has been previously shown, these parasites attack corn roots as readily as those of cane.

In one experiment with sugarcane, plants were grown in pails at different seasons in order to test variations in temperature. One set was started in July and another in October. Pails containing mixed sugarcane soil were autoclaved for two hours at 15 pounds pressure. These were then inoculated by mixing into the soil three-day-old petri dish cultures of Pythium and Rhizoctonia. Three single-eye seed pieces of Louisiana Purple cane were planted in each pail and four pails of each variant were used. Water was added to bring the soil to the required moisture content and the soil was maintained at this moisture content thereafter by weighing regularly. When the plants had about reached their maximum growth in the containers, they were measured and the roots were washed free of soil and examined for decay. The results obtained are given in table 5.

TABLE 5.

THE EFFECT OF MOISTURE ON THE GROWTH OF LOUISIANA PURPLE CANE IN STERILIZED SOIL, INOCULATED WITH PYTHIUM AND RHIZOCTONIA.

Percent moisture-holding capacity of soil	AVERAGE HEIGHT OF STEMS IN INCHES					
	Summer series			Fall series		
	July 18 to Sept. 13, 1927			Sept. 26 to Dec. 11, 1927		
	Check	Pythium	Rhizoctonia	Check	Pythium	Rhizoctonia
25	5.3	5.4	5.5	6.5	3.5	6.7
45	9.0	9.0	8.9	9.1	5.1	9.2
65	8.6	8.5	9.5	8.5	8.7	9.6
85	11.7	9.6	10.3	8.2	6.6	9.0

The data in table 5 show that under greenhouse conditions Rhizoctonia had little effect on the growth of Louisiana Purple cane during either the hot summer months or the cooler months of fall. Pythium had little effect during the summer, but in the fall, the plants in the Pythium inoculated soil averaged somewhat smaller than the controls. The summer temperature in the greenhouse, which was somewhat higher than in the open, evidently retarded the development of the Pythium on the cane roots.

Although inoculating the soil with Rhizoctonia had little effect on the growth of the cane, an examination of the roots showed an abundance of the dark brown lesions. These lesions, however, had not caused any marked reduction in root development.

The effect of Pythium on root development was quite variable. The soft, flaccid root tips characteristic of Pythium rot were present in all soil moisture concentrations in the summer and fall. In the summer series, there was little effect on the total root production, but in the fall series, Pythium reduced the quantity of roots. In the soil kept at 65 percent, the effect was not so noticeable.

Two tests were also made to determine the effect of Pythium on POJ 213 cane. Sterilized soil was used and

the moisture content in different sets was held at 20, 30, 40, 50, 60, 70, 80 and 90 percent of its water-holding capacity. The tests were started in May and July, 1928. The results of the tests are given in table 6.

TABLE 6.

THE EFFECT OF MOISTURE ON THE GROWTH OF POJ 213 CANE IN STERILIZED SOIL INOCULATED WITH PYTHIUM.

Percent moisture-holding capacity of the soil	AVERAGE HEIGHT OF STEM IN INCHES			
	May 2 to June 25, 1928		July 19 to Aug. 17, 1928	
	Check	Pythium	Check	Pythium
20	0.0	0.3	0.0	0.0
30	4.5	4.5	6.3	7.0
40	5.4	6.5	8.5	8.3
50	8.2	8.4	11.0	10.0
60	11.9	10.8	14.7	14.0
70	12.6	11.4	16.6	14.0
80	12.3	11.9	17.3	16.6
90	0.0	0.0	20.1	16.3

During hot weather, Pythium had practically no effect on the growth of sugarcane in the drier soils, while in the wetter soils, there appeared to be a slight, but constant, decrease in growth.

Tests with Corn

To determine the effect of soil moisture and temperature on the development of Pythium root rot of corn, a series of tests was run in the greenhouse in the spring of 1928. Cans of No. 2 size were filled with a mixed cane soil and autoclaved for two hours at 15 pounds pressure. A three-day-old petri dish culture of a parasitic sugarcane Pythium (No. 931) served as inoculum for each can. Ten surface sterilized corn seeds of the variety, Calhoun Red Cob, were planted in each can and water added to bring the soil to the desired moisture content. Four replications of each variant were used. The tests were started

in March, April, May and June, thus giving comparisons of early spring and summer temperatures. The soil temperature in the greenhouse during the test period ranged in March from 56°F to 67 with a mean of 60; in April, from 51 to 72 with a mean of 63; in May, from 65 to 79 with a mean of 71; and in June, from 75 to 88 with a mean of 82. The germination of the seed and the height of the plants were used as criteria for Pythium injury. The results of these tests are given in tables 7 and 8.

TABLE 7.

INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON GROWTH OF CORN IN PYTHIUM INFESTED SOIL.

Percent of water-holding capacity of the soil	AVERAGE LENGTH OF TOPS IN CM.							
	March 1 to 11		April 2 to 21		May 2 to 21		June 8 to 19	
	Check	Pythium	Check	Pythium	Check	Pythium	Check	Pythium
20	5.6	4.3	3.0	3.0	8.5	7.5	4.6	3.3
30	12.2	8.1	10.9	11.9	18.7	16.9	8.3	6.3
40	14.9	9.8	16.5	12.7	26.4	22.3	16.2	13.1
50	17.2	10.8	20.9	12.1	34.0	22.2	23.1	19.9
60	17.8	10.3	25.0	11.6	38.4	19.7	26.7	18.5
70	18.3	8.1	22.6	9.1	36.4	16.3	28.3	18.4
80	18.9	10.0	23.4	8.5	38.8	11.5	29.6	17.2
90	14.5	0.0	20.6	0.0	0.0	0.0	28.6	15.4
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 8.

INFLUENCE OF SOIL MOISTURE AND TEMPERATURE ON GERMINATION OF CORN IN PYTHIUM INFESTED SOIL.

Percent of water-holding capacity of the soil	PERCENT GERMINATION							
	March 1 to 11		April 2 to 21		May 2 to 21		June 8 to 19	
	Check	Pythium	Check	Pythium	Check	Pythium	Check	Pythium
20	12.5	7.5	2.5	10.0	77.5	75.0	12.5	10.0
30	95.0	92.5	87.5	77.5	97.5	90.0	27.5	45.0
40	100.0	95.0	97.5	90.0	90.0	95.0	92.5	80.0
50	97.5	80.0	97.5	85.0	92.5	90.0	97.5	95.0
60	87.5	55.0	80.0	57.5	85.0	77.5	90.0	95.0
70	77.5	37.5	62.5	32.5	80.0	45.0	97.5	95.0
80	70.0	15.0	30.0	20.0	12.5	17.5	85.0	72.5
90	35.0	0.0	12.5	0.0	0.0	0.0	67.5	40.0
100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The data in table 7 show that reduction in growth due to Pythium was most pronounced during the cooler months and in the wetter soils. Soil kept at 20 and 30 percent of its water-holding capacity was too dry, while that at 90 and 100 percent was too wet for the growth of corn. Best growth of corn was obtained in the Pythium series when the soil was kept at 50 percent of its moisture-holding capacity, while in the check series, a moisture-holding capacity of 60 and 70 percent was best.

The injury due to Pythium increased with the moisture content of the soil and was more pronounced at a lower moisture content during the cool months than during the warm months. In March, April, and May, the corn growing in Pythium infected soil kept at 50 percent of its water-holding capacity was only about 60 percent as tall as the check; while in June, it was 85 percent.

The germination results are quite regular except at the extreme moisture variations where the soil was too dry or too wet. Germination was not materially affected in any of the tests in Pythium inoculated soil held at 30 and 40 percent of its water-holding capacity (Figs. 8, 9). In the series conducted during the cool months of March

FIG. 8. Effect of moisture on Pythium root rot of corn. Grown in the greenhouse from May 2 to May 21, 1928, in steamed soil with moisture content ranging from 20 to 80 percent of its water-holding capacity. Of each pair, Pythium inoculated plants on left and control on the right.

and April, there was a noticeable reduction in germination at 50 percent in the Pythium infested soil; in the May series, this reduction was evident at 60 percent; while in June, the 80 percent Pythium infested soil was the first to show a significant reduction in germination. These trials indicate that both the growth and germination of corn in Pythium infested soil are closely correlated with soil moisture and temperature.

To determine more accurately the effect of temperature on the rotting of roots by Pythium, corn was grown in No. 2 metal cans in temperature controlled incubators. The cans were filled with soil and autoclaved for two hours at 15 pounds pressure. They were then inoculated with two similar strains of Pythium, 931 and 1432. Culture 931 had been growing in the laboratory for two years and its virulence was known to be decreasing (16). A single three-day-old petri dish culture on bean pod agar was used as inoculum for each can, this being mixed

FIG. 9. Effect of moisture on Pythium root rot of corn. Grown in the Greenhouse from June 8 to June 19, 1928, in steamed soil with moisture content ranging from 30 to 90 percent of its water-holding capacity. Of each pair, Pythium inoculated plants on right and control on the left.

thoroughly with the soil three days before planting. A sterile plate of bean pod agar was mixed with the soil in the control cans. Five germinating corn seedlings of the variety, Calhoun Red Cob, were planted in each can. Three cans of each Pythium and the same number of checks were grown at each temperature. The soil was maintained at 60 per cent of its water-holding capacity by weighing daily. The results obtained in this test are given in table 9.

TABLE 9.

INFLUENCE OF TEMPERATURE ON GROWTH OF CORN IN PYTHIUM INFESTED SOIL IN TEMPERATURE INCUBATORS.

Temperature ° C	Period of growth	Average length of tops in CM.			Percentage decrease	
		Check	931	1432	931	1432
35	5	24.2	25.8	22.2	6*	8
30	5	30.0	23.5	19.0	22	37
26	5	26.8	15.4	12.0	43	55
23	5	17.4	12.2	10.8	30	38
20	10	30.0	19.0	19.6	37	35
15	20	19.5	10.1	9.3	48	52

*Increase.

Both strains of Pythium were severely parasitic at the intermediate and lower temperatures, but at the higher temperatures, 931 which had been in culture for three years appeared to be less injurious than 1432 which had been in culture only one year. A temperature of 35°C was too high for best growth of corn; but at this temperature, there was very little rotting of the roots (Fig. 10F). At 30°C the corn grew best, although there was considerable Pythium injury. At this temperature, the tops of the corn grown in the Pythium infested soil were appreciably shorter than the controls and the roots were considerably rotted (Fig. 10E). The main roots were discolored and many of the tips had the soft, flabby rot, characteristic of Pythium. There were very few secondary and tertiary roots, and these were rotted severely, being only short protuberances from the main root. With each reduction in temperature from 30° to 15°C, the corn growth was less; and the Pythium injury was more severe (Figs. 10 A-D). At 15°C, even the main roots were rotted off nearly to the seed.

Another form of Pythium injury also appeared on the corn grown at the lower temperatures. In a number of cases, there was a rot of the mesocotyl and the stem above the seed, and the Pythium mycelium was found in the decayed areas. This type of injury was not found on corn grown at temperatures above 23°C. An occasional plant was found partially rotted at 23°C, but at 20° and 15°C, this type of rotting was quite prevalent.

Temperature Studies of Pythium Species in Culture

The effect of temperature on the growth of sugarcane Pythiums in culture was also determined. Portions from the margins of rapidly growing cultures were transferred to petri dishes of Difco dextrose agar and the increase in diameter of the colonies measured at 12-hour intervals. The cultures were grown in incubators with a range of temperature between 15° and 36°C. Eight different cultures of Pythium were used. Four of these, 931, 1432,

FIG. 10. Effect of temperature on Pythium root rot of corn. Grown in temperature controlled incubators ranging from 15 to 35°C. a. Controls, not inoculated, b. and c. Plants from soil inoculated with Pythium cultures, 1432 and 931.

A and B were strongly parasitic cultures isolated from cane in Louisiana. Another, 1285, came from Hawaii, supposedly the organism responsible for the Lahaina disease, a disease similar to the root rot which occurs in Louisiana. Two of the other cultures, C and 1436, while isolated from cane in Louisiana, were only mildly parasitic. The eighth culture, D, was a weak-growing, non-parasitic form. The results are shown in table 10.

TABLE 10.

RATE OF GROWTH OF SEVERAL PYTHIUMS ISOLATED FROM SUGARCANE ROOTS AT VARIOUS TEMPERATURES ON DEXTROSE AGAR.

Temperature ° C	CULTURE DESIGNATION							
	Average Increase in Diameter of Colony in 12-Hour Period							
	931	1432	1285	A	B	C	1436	D
15	1.3	1.1	1.1	1.0	0.9	0.9	0.9	0.7
20	2.1	2.0	1.9	1.8	1.7	1.9	2.1	0.8
25	2.5	2.6	2.6	2.6	2.5	2.5	3.2	1.0
30	2.8	2.9	2.9	2.9	2.9	3.2	4.0	1.1
36	1.4	1.7	2.0	1.7	1.4	2.5	3.2	0.5

All the parasitic strains showed considerable similarity in rate of growth; the rate of growth of all these increasing with the temperature up to 30°C and falling off considerably at 36°C. The slightly parasitic forms had an optimum in the neighborhood of 30°C, but made good growth at 36°C. The non-parasitic form grew slowly at all temperatures. While the differences in the rate of growth of the parasitic forms were slight, the results indicated that culture 931 grew better at the lower temperatures than did 1432; while at the higher temperatures, the reverse was true. These two cultures had been used in inoculation experiments and were known to act in a similar way; 931, however, had been in culture for three years, while 1432 had been grown for only one year.

Relation of H-ion Concentration to Growth

Investigations at the Louisiana Agricultural Experiment Station had shown that parasitic cultures of Pythium could rarely be isolated from cane roots when plated on acidified agar. Many fungi make a satisfactory growth in acid media, and as Pythium seemed to differ in this respect, it was thought desirable to obtain more accurate information as to its growth under conditions of varying H-ion concentration.

The fungus was grown on several different culture media with the hydrogen-ion concentration ranging from 4.6 to 9.6. The results have been published in another article (17). It was found that the fungus would not grow at an initial pH of 4.6 or less. At 5.6, it grew slowly at first, but subsequently caught up with cultures growing at a more favorable hydrogen-ion concentration. It made the most satisfactory growth in culture media with the initial pH ranging from 6.5 to 8.6. At a pH of 9.6, growth started slowly, but at the end of four weeks, the fungus had produced as much mycelium as it did in media with a more favorable hydrogen-ion concentration.

It was also found that the metabolic products of fungous growth changed the pH of the media towards 6.0 regardless of the initial H-ion concentration. This tendency to change the pH towards 6.0 continued as long as the mycelial felt increased in weight. After maximum growth had been reached, the solutions uniformly became alkaline.

As most of the soils in the sugar belt of Louisiana have a H-ion concentration ranging between 6.0 and 7.5, conditions are very favorable for the rapid growth and development of the sugarcane Pythium.

Studies of Physiology of Infection

At it has been repeatedly demonstrated that species of Pythium are able to rot the roots of sugarcane and corn growing in sterile soil, it has been assumed that these fungi can gain entrance to the root without the

assistance of other agencies. In view of the lack of definite information on this phase of the problem, a study was undertaken to determine if possible the manner in which the fungus enters the root, the influence of the age of the root on the penetration of the fungus, and the rapidity of penetration through the root tissues.

Hawkins and Harvey (20) in a study of the parasitism of *Pythium debaryanum* on the potato tuber showed that the fungus secreted a toxin which killed the cells of the potato. It also secreted an enzyme which dissolved the middle lamella, but had little effect on the secondary thickenings. These authors also showed that varietal resistance to the disease was correlated with resistance to mechanical puncture, and concluded that mechanical pressure exerted by the fungous hyphae seemed to be the most important factor in cell wall penetration. Microscopical observations of cell wall penetration by the fungous hyphae seemed to corroborate this theory.

Lee, et al (28), in connection with Pythium root rot of sugarcane in Hawaii state: "Injury from the fungus apparently results from the disintegration of the tissues of the root caused by the toxins given off by the fungus growing in both the cortex and stele of the root. There is very little known of the means of ingress of the fungus into the roots, but apparently in the roots of the Lahaina variety, penetration is possible wthout previous mechanical injury or without the aid of such small animals as nematodes, centipedes, etc."

Preliminary investigations on the physiology of infection showed that there were two types of rots of sugarcane and corn caused by Pythium fungi. One of these, which is caused by the semi-parasitic fungus, produces a cortex rot and does not enter the central cylinder of the root. This fungus kills the cortex cells, but the cells do not collapse; on the contrary, they become hard in contrast to the soft rot produced by the more parasitic Pythium. The lesions produced by this fungus are greyish to a yellowish brown in color. The second type of rot is produced

by the more parasitic Pythium and is of more economic importance in Louisiana. It attacks the central cylinder of the root as well as the cortex, and produces a soft rot with a collapse of the root cells. This fungus is mainly responsible for the soft, flabby root tips and the lack of secondary roots.

To determine how the parasitic Pythium enters and grows in the root tissues, tests were made using roots of corn seedlings. Corn seeds were treated in a 1-400 solution of Uspulun at 52°C for one hour, washed in sterile water, dipped in 95 percent alcohol and flamed, and then germinated on moist filter paper in sterile petri dishes. This treatment rendered most of the seedlings sterile. The rate of penetration of the tissues of the seedling corn root by Pythium was studied in the region of the root just back of the root cap, and in portions that were 16 and 48 hours old. In the study of infection on tissues 16 and 48 hours old, the roots were marked just back of the growing point with India ink 16 and 48 hours respectively, before they were inoculated. Inoculations were made by placing just back of the root tip and at the 16 and 48-hour ink marks one-eighth inch strips of medium and mycelium taken from a three-day-old culture of the fungus on bean pod agar. At intervals of 2, 4, 6, 8, 12 and 24 hours, after inoculation, portions of the root were removed and fixed in a solution composed of 100 cc. of 50 percent ethyl alcohol, 6.5 cc. of formaldehyde, and 2.5 cc. of acetic acid. These were imbedded in paraffin, sectioned, stained in cotton blue, and mounted in gum damar.

Sections made from roots inoculated just back of the root tip showed that infection had been unusually rapid. Within two hours, the Pythium had entered the epidermal cells. In four hours, the first three or four tiers of cells had been penetrated, and in six hours, the fungus had nearly reached the central cylinder. In eight hours, the central cylinder had been reached, but the root tip had not been killed or penetrated. In twelve hours, the central cylinder had usually been penetrated and the fungus hyphae were spreading into the root tip. By twenty-four

hours, the root tip had been completely invaded by the hyphae of the fungus, and the cells had collapsed.

The method of original penetration is not entirely clear but seems to be due to a combination of a disintegration of the outside walls of the epidermal cells and penetration by force. Hyphae have never been observed entering unbroken epidermal cells of a root. The point of entrance is usually where there is a slight depression in the contour of the root. Swollen, knob-like structures, resembling appresoria have been seen at the region where the fungus entered the root, but penetration seems always to be accompanied by the breaking down of the epidermal cells. Once within the root, the fungus spreads out in a fan-like manner, uninfluenced by the cell walls and contents, until the central cylinder is reached. The endodermis and pericycle offer considerable resistance to penetration. Even in the inoculations of the youngest portion of the root, cases were observed in which penetration of the central cylinder had not been effected in 24 hours, although in all cases examined, hyphae had reached the central cylinder in 8 hours. Usually, however, the central cylinder was penetrated by the end of 12 hours and in 24 hours was completely invaded.

Penetration of the cortex tissues that were 16 hours old when inoculated was nearly as rapid as when the inoculation was made just back of the root tip. Within six hours, the hyphae had reached the central cylinder. The central cylinder of these roots, however, checked the growth of the Pythium to some extent. At the end of 12 hours, there was no evidence of penetration and after 24 hours the fungus had gained entrance to only the outer three or four layers of cells of the central cylinder.

The uninjured cortex of roots that were 48 hours old at the time of inoculation was not readily penetrated by the fungus. Even where it did get in, as around the regions ruptured by the protrusion of the secondary roots, growth was exceedingly slow and only the three or four layers of cortical cells immediately adjacent to the secondary root were invaded after 24 hours. The secondary roots were, in

all cases, rotted off to the surface of the primary root; but at the end of 24 hours, the hyphae had not been observed to have gained entrance to the central cylinder of the primary root via the secondary roots. It is evident that most of the infection occurs on the very young tissues.

Discussion

Many ideas and theories have been advanced to explain the failure of certain cane varieties, and it is reasonable to assume that a number of factors are concerned in the problem. Diseases, insect pests, soil fertility and climatic conditions are all factors which must be considered. Also, it must be recognized that the combination of factors responsible for crop failures in one country may not necessarily be the same as in another country.

Among the possible causes for the failure of cane crops is the group of troubles commonly known as root rot, root rot complex, or growth failure complex. In this group are included all factors which may cause a decay or deterioration of the root system or prevent the roots from functioning properly. In this bulletin, the results of investigations on certain phases of the root rot problem have been presented.

It has been shown that the failure of the sugarcane crop has not been due to the running out or deterioration of the old varieties. The yields of from 30 to 35 tons per acre of D74 and Louisiana Purple cane which have been obtained in the Experiment Station fields disprove this idea. Furthermore, it has been shown that seed stalks from stools severely affected with root rot recover when planted in good soil, and produce thrifty plants. Even rhizomes from a severely diseased stool recovered when planted in steamed soil, and produced plants nearly as tall and vigorous as did rhizomes from healthy stools.

Furthermore, no evidence has been obtained to support the theory that growth failure in Louisiana is due to the accumulation in the soil of excessive amounts of salts or toxins. The addition of relatively large amounts of the

sulphate salts of aluminum, calcium, iron (ferric), and magnesium had no appreciable effect on the growth of cane. Cane planted in a soil, taken from beneath stools severely affected by root rot, and repeatedly washed so as to remove soluble salts and toxins, grew no better than did that growing in the untreated soil. However, cane planted in part of this same soil that had been steamed for two hours grew much better than that planted in either the washed or untreated soils.

Since neither senility nor the accumulation of toxic substances in the soil appeared to be important factors in the growth failure problem, it seemed evident that the attack of soil organisms was at least one of the important factors in the problem. Soil organisms which were studied included nematodes, other soil animals, and certain fungi.

Two species of nematodes, whose status in the root rot problem have not yet been definitely ascertained, were found in the roots of sugarcane. One of these, the root knot nematode, *Heterodera radicicola* (Muller), was found only a few times in sandy soil in widely scattered regions of the sugar belt. The other nematode has not yet been identified but resembles *Tylenchus similis* (Cobb). This nematode was found in cane roots in nearly all regions of the sugar belt and in all types of soil. No cases of infestation severe enough to cause serious injury to the cane plants were observed, and it is thought that the nematode factor is not of primary importance.

Certain soil inhabiting animals, such as *Lepidocyrtus violentus*, pit the roots of sugarcane and mutilate the root tips. These animals appear able to injure the growth of cane to some extent on their own account, and when present in soil infested with Pythium, are able to increase the injury caused by the latter. Consequently, they appear to have a bearing on the root rot problem but their actual importance has not been ascertained.

Fungi have long been associated with the root rot disease and a great many different forms have, at one time or another, been reported on decaying roots. Of these, species of Pythium are at present considered to be the most impor-

tant. Earlier investigations had shown that there are a number of species or strains of Pythium which are widely distributed in the cane belt of Louisiana. Certain of the parasitic forms are capable of completely rotting the roots of both cane and corn. The roots become soft and flaccid; and all the tissues, including the central cylinder, are invaded.

Some of the conditions which favor the development and infection with Pythium have now been determined. Root rot of corn and cane by Pythium is favored by lower temperatures and by a high moisture content of the soil. This is in agreement with the general observations on the root rot disease, that it is more severe following cold, wet winters. Excessive moisture in the summer does not cause as much harm as in the winter and spring. These facts emphasize the importance of good drainage, especially during the winter season, and also the importance of removing the dirt from the cane in the spring so that the soil around the roots will become warm.

In regard to soil acidity, it was found that the Pythium grows satisfactorily in solutions with the hydrogen-ion concentration ranging from 5.5 to 9.2. Most of the cane soils in Louisiana come within this range.

Summary

1. The growth failure or root rot complex of sugarcane in Louisiana is not due to the running out or deterioration of the old varieties of cane.
2. No evidence was found to indicate that inorganic salts or soluble toxins are concerned in the root rot problem. Washing the soil had no effect on the growth of sugarcane, but steaming the soil made conditions favorable for a more rapid growth.
3. The root knot nematode, *Heterodera radicicola* (Muller), and a burrowing nematode, similar to *Tylenchus similis* (Cobb), were found in the roots of sugarcane in Louisiana, but never in sufficient quantities to be a major factor in the root rot problem.

4. *Lepidocyrtus violentus* (Folsom), a soil-inhabiting, root-pitting insect slightly injured the growth of cane in steamed soil. In Pythium-infested soil, this insect seemed to increase the injury caused by Pythium.
5. A species of Rhizoctonia produced discolored lesions on the roots of sugarcane but did not appreciably reduce growth.
6. A species of Pythium was severely parasitic on the roots of sugarcane and corn, and was injurious to the growth of the plants.
7. Pythium injury to germination and growth of corn increased with the water content of the soil. Injury was less severe in wet soils in warm than in cool weather.
8. Pythium injury to germination and growth of corn decreased with rise in temperature. At 35°C, Pythium did not injure corn. At 30°C, there was an appreciable amount of injury, and at lower temperatures, injury was severe.
9. In culture solutions, Pythium was sensitive to acidity and tolerant to alkalinity. It did not grow in solutions of pH less than 5.2 or greater than 10.0. It grew well within the pH range of 5.5 to 9.2.
10. The semi-parasitic Pythium produced a cortex rot and did not enter the central cylinder. The cortical cells were killed and became hard. The lesion became greyish to yellowish in color.
11. The parasitic Pythium invaded all the tissues of the young roots and produced a soft rot with a collapse of the root cells.
12. The fungus appeared to enter the young roots through a combination of the disintegration of the outside walls of the epidermal cells and penetration by force. Once within the root, the progress of the fungus was rapid, seemingly, uninfluenced by the cell walls until the central cylinder was reached. The

central cylinder offered considerable resistance to invasion; but in young roots, it was usually overrun by the fungus in 24 hours.

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